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Courtship and mating behaviour of manta rays *Mobula alfredi* and *M. birostris* in the Maldives

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ABSTRACT

Manta rays *Mobula alfredi* and *M. birostris* are among the most conspicuous and charismatic of the elasmobranchs, however their courtship and mating behaviour is rarely observed. Although the mating stages of manta ray reproduction have been described, the full detail of their elaborate courtship has not. The aim of this fourteen year study was to elucidate the entire courtship and mating behaviour of both manta ray species using behavioural observations, video and photographic records. From 2003 through 2016, over 11,000 surveys were undertaken at known manta ray aggregation sites in the Maldives to record any observed manta ray reproductive activity. A total of 47,591 photo-ID sightings of 4,247 individual *M. alfredi* and 229 photo-ID sightings of 226 individual *M. birostris* were recorded at 22 atolls and across 265 different sites. Courtship activity was observed on 206 surveys at 30 different sites. A total of 229 courtship events were recorded, with 90% (n=205) of them occurring at cleaning sites. The observed courtship activity was categorised into seven distinct stages which are described in detail: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulatory positioning, (5) copulation, (6) post-copulatory holding, and (7) separation. Photographs provide the first scientific record of the entirety of manta ray courtship and mating. Both *M. alfredi* and *M. birostris* appear to engage in the same elaborate courtship rituals, exhibiting the same behaviours during all stages of the courtship and mating process.

Key words: cleaning stations, copulation, courtship trains, mate choice, reproductive behaviour

INTRODUCTION

Although much is known about the reproductive anatomy and different modes of elasmobranch reproduction (Wourms, 1977; Dodd, 1983; Gilmore, 1993; Wourms & Demski, 1993; Conrath & Musick, 2012), shark and ray reproductive behaviour is poorly understood, mainly due to the difficulties of observing natural behaviour in free-living animals and the artificial nature of studies in captivity (Whitney et al., 2004). Most recently, Pratt and Carrier (2005) summarised the published literature on elasmobranch reproductive behaviour, but their work represented just a small fraction of the total species pool. In their study, many reports came from captive animals and little photographic documentation was provided.

Manta rays are highly conspicuous and charismatic elasmobranchs. Until 2017 these zooplanktivorous rays were separated from other mobulids in their own genus: *Manta* (Bancroft, 1829). However, the Mobulidae family is now considered monogenetic, comprising nine species in the Genus *Mobula* (Rafinesque, 1810; White et al., 2017). In 2009, the monospecific *Manta* was split into the two species of manta ray currently recognised (Marshall et al., 2009): the reef manta ray *Mobula alfredi* (Krefft, 1868) and the oceanic manta ray *Mobula birostris* (Walbaum, 1792). *Mobula alfredi* is widely distributed throughout the tropical and sub-tropical waters of the Pacific and Indian Oceans, although populations appear to be highly fragmented (Kashiwagi et al., 2011; Couturier et al., 2012); most likely due to resource and habitat requirements (Anderson et al., 2011*a*). *Mobula alfredi* frequent the coastal reefs of continents and remote oceanic islands (Kashiwagi et al., 2011; Marshall et al., 2011*a*), but also venture offshore into the mesopelagic zone (Braun et al., 2014; Jaine et al., 2014). *Mobula birostris* is distributed throughout the tropics, and within waters up to 41° of latitude (Kashiwagi et al., 2011). It is thought that *M. birostris* spend the majority of

66 their time offshore, but come into shallower regions along productive coastlines with regular
67 upwellings (Marshall et al., 2011*b*; Stewart et al., 2016*a*). *Mobula birostris* also aggregate
68 around oceanic island groups and offshore pinnacles, seamounts and submarine ridge
69 systems, where they are known to engage in courtship (Compagno & Last, 1999; Yano et al.,
70 1999; Rubin, 2002; Marshall et al., 2011*b*; Stewart et al., 2016*b*).

71 Manta rays are ovoviviparous matrotrophs (Wourms, 1977; Dulvy & Reynolds, 1997) and
72 like all elasmobranchs, employ internal fertilization (Conrath & Musick, 2012). The gestation
73 time of *M. alfredi* is reported to be one year (Marshall & Bennett, 2010; Okinawa Churaumi
74 Aquarium, 2010; Deakos, 2011; Stevens, 2016), but remains unknown for *M. birostris*.
75 Reproductive cycles often include resting periods, with biennial reproduction reported as the
76 norm for individual *M. alfredi* within populations in Hawaii and Mozambique (Marshall &
77 Bennett, 2010; Deakos, 2011). Inter-birth intervals of several years or more are common in
78 matrotrophs which also invest heavily in post parturition parental care, such as Sumatran
79 orangutans *Pongo pygmaeus abelii*, capuchins *Cebus capucinus*, African and Asian elephants
80 *Loxodonta africana* and *Elephas maximus*, and bottlenose dolphins *Tursiops sp*. (Lee & Moss,
81 1986; Fedigan & Rose, 1995; Mann et al., 2000; van Noordwijk & van Schaik, 2005; Robinson
82 et al., 2012), but are less common in species which do not. Amongst species which do not
83 undertake post parturition parental care, biennial and triennial reproductive cycles have only
84 been reported in reptiles (Cree & Guillette, 1995; Ibargüengoytía & Cussac, 1996; Sever et al.,
85 2000) and elasmobranchs (Colonello et al., 2006; Whitney & Crow, 2006; Castro, 2009). As a
86 general point, rest periods between reproduction are thought to occur to allow females to
87 recuperate energy reserves (Catry et al., 2006; Trinnie et al., 2012). In manta ray reproduction,
88 females normally give birth to a single, large pup (Coles, 1916; Beebe & Tee-Van, 1941;
89 Bigelow & Schroeder, 1953), although rare cases of twins have been recorded (Marshall &

Bennett, 2010), with size at birth ranging from 130 – 190 cm in disc width (Marshall & Bennett, 2010; Okinawa Churaumi Aquarium, 2010).

Manta ray reproductive behaviour in the wild has rarely been observed and virtually all documentation that exists is for *M. alfredi*, with just one record of mating for *M. birostris*. This was recorded off the Ogasawara Islands, Japan in 1997 and describes a female copulating with two different males on the same day (Yano et al., 1999). For *M. alfredi*, most courtship and mating reported from the wild has been at manta feeding and cleaning sites (Marshall & Bennett, 2010; Deakos, 2011). The mating events number just five (Marshall & Bennett, 2010) and all described females mating once, with one male.

Field observations in Japan, Mozambique and Hawaii indicate the same complex mating process occur in both manta ray species, and that this involves a five-step sequence of (1) chasing, (2) biting, (3) copulating, (4) post-copulation holding and (5) separation (Yano et al., 1999; Marshall & Bennett, 2010; Deakos, 2011). Copulation occurs when the female slows to allow a pursuing male to position himself directly on top of her dorsal surface. At this point the male slides his mouth down the side of the female's body, nearly always her left, to the tip of her pectoral fin, before taking about a metre of this fin into his mouth. The male then bites down hard to gain leverage on the female's body, twisting underneath her so that the female's ventral surface is in alignment with his, enabling him to insert a clasper into her cloacal opening before releasing his seminal fluid (Yano et al., 1999; Marshall & Bennett, 2010).

Although observations of actual copulation are extremely rare, pre-copulatory chasing has been more commonly observed, especially in *M. alfredi,* where multiple escorting males pursue a single, fast swimming female in what is commonly termed a 'mating train' (Marshall & Bennett, 2010; Deakos, 2011). This behaviour, known as 'female recruitment runs' appears

to be the basis of pre-copulatory mate choice by females and can last for hours (Whitney et al., 2004; Deakos, 2011). However, as it does not always result in mating (Stevens, 2016), and appears to be driven primarily by female mate choice, this behaviour hereafter is referred to as a 'courtship train'.

During courtship trains, the female initiates high speed flips, turns and somersaults, while pursuing males mimic her evasive manoeuvres (Marshall & Bennett, 2010; Deakos, 2011). Because several different behaviours appear to take place within step one of the courtship classification described by Yano et al. (1999), Marshall and Bennett (2010) proposed this step should be broken down into three subdivisions namely: (1) following or chasing, (2) a complex series of interactive turning and flipping performed by both the female and her suitors, and (3) evasive or avoidance behaviour exhibited by the female.

Courtship trains have been observed in several close relatives of manta rays, namely in: flapnose ray *Rhinoptera javanica*, cownose ray *Rhinoptera bonasus,* spotted eagle ray *Aetobatus narinari,* spinetail devil ray *Mobula mobular* and sicklefin devil ray *Mobula tarapacana* (Tricas, 1980; Uchida et al., 1990; pers. obs.). Whitetip reef sharks *Triaenodon obesus* and nurse sharks *Ginglymostoma cirratum*, also display the 'mating avoidance' shown in manta rays whereby a female 'arches' her body during attempted copulation by males to keep her cloaca out of their reach (Pratt & Carrier, 2001; Whitney et al., 2004).

There are also parallels between the courtship trains of manta rays and the tending behaviour undertaken by ungulates, where a male will associate with an oestrous female until he either copulates with her or is displaced by another male (Vos et al., 1967; Kucera, 1978; Wolff, 1998; Mysterud et al., 2004; Byers et al., 2005). In marine mammals similar behaviour has been observed in humpback whales *Megaptera novaeangliae* (Tyack & Whitehead, 1982; Baker & Herman, 1984; Spitz et al., 2002; Herman et al., 2007).

While the major stages of manta ray mating have already been described, this study provides new detail about the process; the aim being to elucidate the entire courtship and mating behaviour of both manta ray species using behavioural observations backed up by video and photographic records.

METHODS

STUDY AREA AND PERIOD

Field research was undertaken in the Maldives in the Indian Ocean, where the world's largest known population of *M. alfredi* (Kitchen-Wheeler et al., 2011; Stevens, 2016) occurs and where *M. birostris* is known from several locations where remote seamounts rise from extremely deep water (Kashiwagi et al., 2011; Stevens, 2016). The 26 coral atolls which form the Maldives archipelago extend from 7° north down 870 km to half a degree south of the equator (Fig. 1). During a fourteen year study from 2003 through to the end of 2016, 11,088 surveys throughout the Maldives were undertaken at known aggregation sites for *M. alfredi* and *M. birostris* to observe and photographically record their predominant behaviours and to specifically document activity related to courtship and reproduction. At each site surveyed, the predominant behaviour of all observed manta rays was recorded. In total 64 (24%) of the sites surveyed were considered to be primarily used as cleaning sites by the manta rays (Losey Jr, 1972; Côté, 2000; O'Shea et al., 2010; Jaine et al. 2012), 96 (36%) as feeding sites (Dewar et al., 2008), and 105 (40%) sites where manta rays mostly travelled through the area.

SAMPLING PROTOCOL

In the Maldives, manta rays are accustomed to interacting with tourist divers and snorkellers at aggregation sites where the rays predictably gather at certain times of the year to feed, clean and socialise (Anderson et al., 2011*b*; Stevens, 2016). A typical survey during this study entailed diving or freediving at one of these aggregation sites, where close encounters with the unperturbed rays easily allowed photo-ID images to be taken and observations recorded of the individuals present (Marshall & Pierce, 2012; Stevens, 2016). Manta ray surveys were performed from either a dedicated research vessel or commercial diving vessels. Surveys were performed at different times of day throughout all months of the year. SCUBA surveys lasted on average 60 minutes and ranged to a maximum depth of 30 metres. Freediving surveys lasted on average 120 minutes. The first author, or a trained staff member or volunteer from the Manta Trust (www.mantatrust.org), conducted the surveys (Stevens, 2016).

When a manta ray was encountered, it was photographed and/or videoed and its: (1) species, (2) sex, (3) pregnancy status if female, (4) maturity status, and (5) behaviours exhibited were recorded. Behavioural activity was distinguished into: (1) feeding, (2) cleaning, (3) cruising, and (4) courtship. The activity which dominated the encounter was recorded as the primary behaviour. Only courtship behaviour is considered further here.

The observed courtship activity was categorised using a methodology developed during this study after initial observations found that the courtship and mating stages proposed by Yano et al (1999) and Marshall and Bennett (2010) did not accurately encompass, or define, all of the behaviours observed. As a result, the following seven distinct courtship stages are identified: (1) initiation, (2) endurance, (3) evasion, (4) pre-copulatory positioning, (5) copulation, (6) post-copulatory holding, and (7) separation (Table I).

Given the scarcity of courtship or mating behaviour reported in the literature, and the rarity of observing these events in the wild, an extensive search of the online search engine *YouTube* (www.youtube.com) was performed in an attempt to gather further observational data to supplement this study. This online data was also used to ensure the sequence of behaviours described in this study were consistent across sites and populations. Using different combinations of the key words 'manta', 'mating', 'courtship' and 'copulation' the search engine produced results for ~8,000 videos. All resulting videos containing relevant information were viewed (~150) and the manta ray behaviour exhibited within them recorded using the same protocol as field observations.

RESULTS

In total 47,591 photo-ID sightings of 4,247 individual *M. alfredi* and 229 photo-ID sightings of 226 individual *M. birostris* were made from 22 atolls at 265 sites in the Maldives. Courtship activity was observed on 206 surveys at 30 different sites (Table II). Although it was not possible to identify every individual involved, 420 different *M. alfredi* (143 females and 277 males) and six *M. birostris* (two females and four males) were individually identified by their unique ventral spot markings after comparing these to existing databases (Marshall & Pierce, 2012; Stevens, 2016). Over 14 years, a total of 229 courtship events were recorded, with 90% (n=205) occurring at cleaning sites. All but two courtship events involved *M. alfredi* (Table III).

Using the seven stages of manta ray courtship behaviour described in the methodology, 73 instances of initiation (stage 1) were observed, 168 of endurance (stage 2), 40 of evasion (stage 3), two of pre-copulation positioning (stage 4), one of copulation (stage 5), no post-copulation holding (stage 6) and one separation (stage 7). Separation without post-copulation

holding (stage 6) occurred because in the one case where copulation was observed the couple separated immediately.

Only two courtship events were observed for *M. birostris*; the first involved eight males in a train behind one female; and the second just one male and one female. For *M. alfredi,* of the 73 initiation events only 12 (16%) advanced to the endurance stage of a courtship train. The number of males involved in these varied greatly, with a minimum of only one male chasing a single female, to a maximum of 26 males in the train. The average ratio of males-to-females in a single courtship train was 3:1 (SE ± 0.2, n = 168), although this rose to 5:1 (SE ± 0.7, n = 40) if the courtship escalated to the evasion stage. On 12 occasions, two females were involved in a single train (Table III). During these events the second of the two females was usually directly behind the lead female, although their positions in the courtship train sometimes varied during the encounter. Based on their highly distended abdomens, a total of 29 of the females (12% of the total) observed engaging in courtship during the study were near-term pregnant individuals, while a further 23 females (10% of the total) observed engaging in courtship activity had fresh mating wounds.

The courtship and mating events recorded in this study have allowed a clearer understanding of manta ray behaviour to emerge, specifically as a result of several noteworthy events amongst the observations. These events are described below in the sequence in which manta ray courtship and mating occurs from specific examples recorded in this study (Table III).

STAGE ONE (INITIATION)

Event 136: 06/04/2015 – Rasfari North, North Malé Atoll

At 9:57 am while four adult *M. alfredi* were observed to circle the site's cleaning station, an approaching male swam straight towards one of the females being cleaned. Manoeuvring himself directly above her, he unfurled his cephalic fins and placed them onto the female's head (Fig. 2). The female reacted by rapidly raising her body forcefully into the male's ventral surface, physically pushing him backwards. This upward thrust was followed by a flick of one pectoral fin in what appeared an attempt to dislodge the male. This action resulted in the displacement of the male from the female's back and cessation of further courtship.

STAGES TWO (ENDURANCE) AND THREE (EVASION)

Event 59: 08/11/2008 – Lankan Beyru, North Malé Atoll

The event began at 9:24 am with a repeat of the behaviour described above, except this time the female reacted to the male's presence directly above her by rapidly swimming forwards along the reef. The male followed, attempting to maintain his position on top of the female's back. The ensuing courtship train was observed for several minutes while the pair remained within sight along the reef crest between 5 – 20 metres deep. Periodically the female undertook several forward flips and backward somersaults, while the male, shadowing her movements, remained within one or two metres at all times (Fig. 3). Between flips and somersaults the female swam faster than is usual, and made several quick changes in direction while the male stayed close. During this encounter fresh mating wounds were visible on the tip of the female's left pectoral fin, indicating she had recently mated (Fig. 3 circled).

Event 39: 07/11/2007 – Lankan Beyru, North Malé Atoll

This event spanned two dives which were both approximately 60 minutes long. 16 minutes into the first dive at 07:16 am, while observing three adult female *M. alfredi* at the main

253 cleaning station on the reef at 20 metres depth, another female swam rapidly overhead
254 followed by 26 males in a courtship train. Due to the large number of males their trailing line
255 was less delineated than it would be in a more typical chain of several males. Over the next
256 fourteen minutes the courtship train remained within sight and, as in event 59, the female
257 undertook multiple tight turns, forward flips and backward somersaults. Often she would loop
258 back on herself to slot behind the following males, causing apparent confusion, resulting in
259 an unstructured group around the cleaning station. The female also appeared to chase
260 individual males at times, closely following one of the leading males in the courtship train
261 while multiple other pursuing males attempted to position themselves onto her dorsal
262 surface. Each time one of the pursuing males succeeded in getting within touching distance
263 of her back she would perform another series of forward flips, or backward somersaults. After
264 14 minutes the female rapidly swam off followed by the train of males, at which point
265 observations ceased until the next dive (Fig. 4). At 11:44 on the second SCUBA dive, the same
266 female from the previous courtship train again swam along the reef crest at a depth of 15
267 metres, this time pursued by eight males. Six were later identified as present at the earlier
268 courtship event. The courtship train passed quickly and remained in sight for only three
269 minutes. The total time between first and last sighting of this courtship train was four hours
270 and 31 minutes.
271
272 *Event 51: 20/09/2008 – Hanifaru Bay, Baa Atoll*
273 This event took place at 15:26 while the observers were free-diving inside Hanifaru Bay
274 and involved five males and a single near-term pregnant female. The observation lasted two
275 minutes. The pregnant female swam rapidly into the vicinity of the observers, where visibility
276 was only 12 metres, followed by the five males in a courtship train. In ten metres of water the

277 female undertook four tight backwards somersaults while the following males attempted to
278 maintain position close behind, or directly on top of the female's dorsal surface (Fig. 5). After
279 the last somersault the female rapidly swam out of sight followed by all of the males.
280
281 *Event 88: 06/06/2013 – Hurai Faru, Baa Atoll*
282 A courtship event similar to event 50 at Hanifaru Bay occurred several years later at the
283 nearby *M. alfredi* feeding site of Hurai Faru, in which a female was engaged in a courtship
284 train with three males. The female employed similar evasive flipping and somersault
285 behaviour, although in this instance on two occasions she intentionally swam at increased
286 speed within a metre of the freediving observers. On the second of these approaches the
287 female, pursued by the three males, swam directly towards one observer, leaping clear of the
288 water before landing partially on top of the observer, who was filming. The footage can be
289 viewed at: https://youtu.be/9tpkVjcxqK8.
290 During this event the female also actively switched from being pursued, to following one
291 of the males for approximately 30 seconds after a series of flipping manoeuvres which
292 resulted in her looping back. Similar female pursuit behaviour was also observed in three
293 other events (Nos. 63, 84 and 105).
294
295 *Event 77: 08/04/2012 – North Point, Fuvahmulah Atoll*
296 During the study only two courtship events involved *M. birostris*. Both occurred at the
297 remote atoll of Fuvahmulah in the far south of the Maldives, a known aggregation site for the
298 species. During this event one large female (disc width ~5 metres) was chased by eight smaller
299 males (average disc width ~4 metres) along the reef crest of the northern tip of the atoll. The
300 observation lasted only a minute, but the behaviour was similar to courtship trains recorded

for *M. alfredi*. The second *M. birostris* event (No. 135) also lasted one minute and involved one male chasing a single female along the Southern Spur Reef of the atoll.

STAGE FOUR (PRE-COPULATION POSITIONING)

Event 2: 06/01/2004 – Rangali Madivaru, Ari Atoll

This event occurred on the shallow reef crest in water three metres deep close to another well-known *M. alfredi* cleaning station frequented by tourists. At 15:20 the sight of a leaping manta ray and the resulting splash alerted observers to the presence of several *M. alfredi* and these were followed by snorkel for the next three minutes. The courtship event involved a lead female and two males. When first spotted, the two males were attempting to position themselves onto the dorsal surface of the female, which remained almost motionless one metre above the reef (Fig. 6a). Both the male's mouths were open as each physically competed to engulf her left pectoral fin (Fig. 6b – d). After 60 seconds one of the males succeeded, whereupon he rotated and flipped his body underneath the female's to align his abdomen against hers (Fig. 6e – h). Throughout this process the female remained motionless, while the other male carried on trying to gain purchase on the female's left pectoral fin, using his head and body to ram the successful male which remained firm (Fig. 6g).

The lack of forward swimming motion by the three negatively buoyant manta rays, and the very shallow water, caused all three to sink onto the reef (Fig. 6i). Upon collision the male holding the female released his grasp, allowing her to rapidly swim off with both males in pursuit. Copulation was not seen.

STAGES FIVE (COPULATION) AND SEVEN (SEPARATION)

Event 1: 19/11/2003 – Lankan Beyru, North Malé Atoll

Observers encountered a single male *M. alfredi* following a female at 10:52 am at 20 metres depth on SCUBA. The manta rays were swimming rapidly along the reef crest at approximately 15 metres depth close to the nearby cleaning station where several other manta rays were being cleaned. The female's swimming behaviour was erratic; making tight twists and turns, she swam directly at the observers, passing within less than a metre, while the male manta ray maintained a position less than two metres behind the female at all times. The pair quickly disappeared from view, swimming too fast to be followed. Five minutes later they re-appeared from the direction in which they had departed with the female in the lead. Their swimming speed was now reduced to normal cruising and erratic movements had ceased. When the manta rays were parallel to the observers the male drew closer to the female, positioning himself directly on to her dorsal surface (Fig. 7a). The female reacted by slowly swimming up, at which point the male began to slide his open mouth down the leading edge of her left pectoral fin, using his cephalic fins to guide the tip into his mouth; engulfing one metre, the male then grasped hold of the fin (Fig. 7b – d). The female ceased swimming while the male flipped his body underneath her, abdomen to abdomen in the water column within 10 metres of the surface. The male then inserted a clasper into the female's cloacal opening, while continuing to slowly beat his pectoral fins (Fig. 7e). He made rapid pelvic thrusts which lasted for 10 seconds as the copulating pair slowly spiralled clockwise while sinking. Copulation lasted for approximately 30 seconds before they separated and swam off in different directions. Removal of the male's clasper resulted in a small milky cloud of fluid, presumably sperm, released from the female's cloaca. This action occurred simultaneously with the male releasing his grip on the female's pectoral fin. No post-copulation holding occurred.

Supplementary on-line observational footage

An internet search on the video platform *YouTube* revealed ten manta ray courtship and mating event videos which show behaviour rarely observed (Table IV). Footage came from a wide variety of locations and for both species provided rarely seen copulation (Table IV, events one, two and ten). In all three, as in the copulation observed in this study, there was no post-copulatory holding by males, with copulation ceasing simultaneously when the male released his grip on the female's pectoral fin. Event two of the online observations records behaviour very similar to the mass courtship event observed during this study (event 39). Online events three and five (Table IV) recorded near-term pregnant females engaged in courtship behaviour that is consistent with the 28 courtship events in the Maldives where 29 near-term pregnant females were engaged in initiation, endurance and evasion behaviours. The online event number nine (Table IV) also documented initiation behaviour for *M. birostris*, which means the complete sequence of courtship and mating behaviour for both species has now been observed and documented. All other online courtship and mating behaviour recorded in Table IV is consistent with observations recorded in the field in the Maldives in this study.

DISCUSSION

For the first time a detailed photographic record of manta ray courtship and reproductive behaviour is presented, with the observations collated here adding significant new detail to previous reports in the scientific literature. Seven distinct stages to the courtship and mating process in manta rays are identified, which occur within both species (Fig. 8).

371 Our study largely agrees with both Yano et al. (1999) and Marshall & Bennett (2010), but
372 advances their work by more accurately defining manta ray courtship and mating and by
373 describing a new stage (initiation). We also consider that Marshall and Bennett's sub-division
374 of 'turning and flipping' and 'evasion or avoidance' should be combined into a single category
375 because turning and flipping is the action taken by females trying to evade or avoid males.
376 Although male manta rays appear to initiate courtship, it is possible that females could
377 trigger courtship by releasing olfaction-mediated pheromones indicating readiness to mate
378 (Johnson & Nelson, 1978). This could explain the close following and courtship train behaviour
379 observed in manta rays and other elasmobranchs (Klimley, 1980; Tricas, 1980; Luer & Gilbert,
380 1985; Gordon, 1993), although no experimental evidence is available to support this
381 hypothesis (for review also see Demski, 1991). By contrast, the use of sex pheromones to
382 attract mates in the wider animal kingdom is well documented (Wyatt, 2003; Hurst, 2005).
383 Several compelling accounts of proposed olfaction-mediated sex attraction in black-tip
384 reef sharks *Carcharhinus melanopterus* have been reported from French Polynesia (Johnson
385 & Nelson, 1978). One of these accounts involved one shark tracking down another (which was
386 initially out of view), then following it closely with the snout directed towards the leader's
387 vent. Very similar behaviour in manta rays was also observed in French Polynesia during a
388 courtship event involving a near-term pregnant female *M. alfredi* and three males (M. de
389 Rosemont, pers. comm.). The four manta rays were initially engaged in a courtship train above
390 a cleaning station, but after several minutes of observations the fast-moving manta rays
391 moved out of view. However, a few minutes later the female returned, now alone, swimming
392 at above average speed along the reef in a straight line. The female continued on the same
393 course until reaching the cleaning station, at which point she changed course and headed into
394 deeper water away from the reef, disappearing from view again within 15 seconds. During

the next 60 seconds, all three of the males which had been following the female returned one by one. Travelling in the same direction as the female, they swam along the reef in a zig-zag motion with cephalic fins unfurled. Upon reaching the point where the female changed course each of the males appeared to sense the direction in which she had departed as all altered course to head seaward at precisely the same point she did. Similar behaviour by male *M. alfredi* was observed during this study in the Maldives, although the event described above provides the strongest circumstantial evidence to support the hypothesis that olfaction-mediated cues are important in manta ray courtship and mating.

Further supporting the use of olfaction-mediated cues, a study on captive sandtiger sharks *Carcharias taurus* by Gordon (1993) suggested the observed action of flaring and cupping of the pelvic fins by females during courtship may serve as a pumping action, excreting a chemical stimulant (pheromone) into the water, attracting nearby males. During courtship event 39 in this study (discussed above), the pursued female excreted an almost clear liquid into the water from her cloacal opening during the courtship train, repeatedly everting her intestine in the process to pump the discharge into the water (Clark et al., 2007) (Fig. 9a). This was quite unlike the cloudy mass of reddish-brown material which manta rays produce when defecating (Fig. 9b) (pers. obs.). After the female released the clear liquid, her pursuing males' sped towards her and attempted to position themselves closely behind her. In response she then exhibited evasive behaviour.

During courtship trains the female's cephalic fins usually remain tightly rolled up, while the males are usually unfurled. These modified head-fins are primarily used by manta rays during feeding where they funnel planktonic food into their mouths (Paig-Tran et al., 2013; pers. obs.). When manta rays are 'cruising' these fins are curled up, presumably to improve hydrodynamic efficiency. Manta rays have two small nostrils, the outer openings of which are

situated on the upper jaw either side of their mouth. The nostrils are aligned so that while the manta ray swims forward water flows into them, passing over sensory folds, allowing the manta rays, like other elasmobranchs, to detect tiny concentrations of dissolved chemicals (Theisen et al., 1986; Zeiske et al., 1987). Thus, if female manta rays release sex pheromones during courtship trains, following male manta rays could maximise their sensitivity by unfurling their cephalic fins to increase water flow to their nostrils.

The endurance stage of manta ray courtship consisted on average of a 3:1 (SE ± 0.2, n = 168) ratio of males to females, rising to 5:1 (SE ± 0.7, n = 40) if the courtship train escalated to the evasion stage. Female manta rays and elasmobranchs in general invest heavily in their offspring, while males invest little (Conrath & Musick, 2012). This means that females are more likely to be selective in mate choice (Trivers, 1972; Bleu et al., 2012), thereby driving contest competition among males (Cox & Le Boeuf, 1977), and explaining the female evasive behaviour observed in manta rays and other elasmobranchs (Whitney et al., 2004; Pratt & Carrier, 2005; Deakos, 2011). In manta rays, females can engage in multiple courtships trains and determine their speed, duration and direction, which can last for hours and may not result in copulation. Indeed, given that 12% (n=29) of female *M. alfredi* observed engaging in courtship during this study were near-term pregnant at the time, females appear to regularly engage in courtship activity before they are ready to copulate. Similar courtship behaviour by near-term pregnant females from four other mobulid species (*M. birostris, M. mobular, M. kuhlii* and *M. tarapacana*) has also been observed and appears to be a common reproductive strategy employed by this family of rays (Stevens, 2016; Duffy & Tindale 2018; pers. obs.). However, the fact that this study found regular occurrence of fresh mating wounds on females that were not visibly pregnant but engaged in courtship trains suggests that multiple matings as a result of multiple courtship events do occur, and are not uncommon.

443 A courtship train may on occasion also involve two females. During these events the lead
444 female is usually followed closely by the second which appears to be deliberately following
445 her, while males trail behind. It is unclear why this behaviour occurs, but if multiple sexually
446 receptive females are present in an area, a passing courtship train may attract additional
447 females, as the train of males provides a ready source of potential suitors for the joining
448 female. Furthermore, the presence of two females engaged in a single courtship train doubles
449 the chances of each male successfully copulating, which potentially should attract more
450 males. Ninety percent of the courtship events recorded during this study occurred at cleaning
451 stations, raising the possibility that they may also function as leks for manta rays (Stevens,
452 2016). These sites appear to create a focal point for courting animals, where individuals can
453 join passing courtship trains to assess or compete for prospective mates (Beehler & Foster,
454 1988).
455 Tonic immobility is known from many ray and shark species and may help induce females
456 to copulate and reduce risk of injury during copulation (Whitman et al., 1986; Henningsen,
457 1994). Manta rays are sensitive to touch (pers. obs.) and it is possible that tactile stimulation
458 serves as a way for males to pacify a female and trigger the onset of copulation, during which
459 a female enters a passive, almost hypnotic state. The dorsal surface of manta rays is covered
460 by a layer of mucus which contains dark pigmentation, creating darker shading where the
461 mucus layer is thickest. The layer is easily rubbed off through light contact (pers. obs.). During
462 courtship the males' unfurled cephalic fins rub the back of the female's head or pectoral fin,
463 revealing a lighter skin tone underneath. These marks quickly darken and the natural skin
464 pigmentation returns within a few weeks, unlike the permanent scars on the dorsal surface
465 of the females' pectoral fin tips which can arise from mating.

466 At the onset of pre-copulation positioning, whether tactile stimulation plays a role or not,
467 something causes the female to reduce her swimming speed and cease evasive behaviour,
468 allowing the male to grasp her pectoral fin. As the majority of copulation events observed
469 consisted of just a single male and female, the lengthy duration of courtship trains may
470 constitute a form of control by females to selectively reduce competing males until only the
471 most persistent remains, similar to the heat runs exhibited by humpback whales *M.*
472 *novaeangliae*, where the principal escort (male) attempts to hold his position next to the
473 female throughout her late pregnancy (Baker & Herman, 1984; Herman et al., 2007). In this
474 way females may ensure only the fittest males are selected as a mate.
475 During our study, near-term pregnant females were regularly involved in courtship trains,
476 suggesting that females are likely to mate soon after giving birth. Indeed, fresh mating
477 wounds were recorded on females soon after parturition, although any subsequent gestation
478 often appears to be delayed for many months or even years in the wild (Stevens, 2016). These
479 field observations are supported by the mating behaviour of a female *M. alfredi* held in
480 captivity in Okinawa Churaumi Aquarium, Japan (Okinawa Churaumi Aquarium, 2010). From
481 four consecutive pregnancies this individual gave birth to a single pup then copulated within
482 hours of parturition. To date, these are the only observations of any manta ray giving birth.
483 Protracted courtship may also increase reproductive success in other ways. Females that
484 allow copulation while multiple males are still engaged in courtship activity with her run the
485 risk of injury through collision with the reef. The presence of multiple males during the later
486 stages of courtship is also likely to reduce the chance of a successful copulation as competing
487 males prevent each other from successfully positioning themselves for penetration. Previous
488 mating experience of a female may also play a role in the timing of the pre-copulation
489 positioning trigger.

Post-copulation holding behaviour was observed in six of the seven previously described manta ray mating events (Yano et al., 1999; Marshall & Bennett, 2010). However, of the four documented copulation events in this study, no post-copulation holding was observed, with separation commencing simultaneously upon cessation of copulation. It is unclear what benefit, or significance, may be derived from this post-copulatory behaviour, therefore future studies will need to determine if it warrants the current stage categorisation.

In summary, this study confirms that both *M. alfredi* and *M. birostris* appear to engage in the same elaborate courtship rituals, exhibiting similar behaviours during all stages of the courtship and mating process. These courtship rituals are most prevalent at cleaning stations in *M. alfredi*, which may also function as lekking sites (Stevens, 2016). The study also suggests female manta rays invest heavily in mate choice, thereby shaping their reproductive strategies.

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